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FULL PAPER

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In vitro cytotoxic and apoptotic effect of *vic*-dioxime ligand and its metal complexes

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In this study, *vic*-dioxime ligand, (1E,2E)-2-(hydroxyimino)-*N*′-[(1E)-2-oxo-2-phenylethylidene]ethanehydroximohydrazide (LH_2), and its Cu (II) and Ni (II) transition metal complexes were synthesized and characterized using analytical and spectroscopic techniques. Furthermore, *in vitro* cytotoxic and apoptotic effects of this *vic*-dioxime ligand and its Cu (II) and Ni (II) complexes on Caco-2 heterogeneous human epithelial colorectal adenocarcinoma cells were evaluated. The effect of the *vic*-dioxime ligand and its Ni (II) and Cu (II) complexes in combination with Campto on the cells was also investigated. The cytotoxicity test was carried using the MTT assay, and the apoptotic effect was tested by DNA diffusion assay. Campto was used as a standard anti-cancer drug, Caco-2 cancer cells treated with dimethylsulfoxide acted as solvent control, and human peripheral lymphocytes were used as control.

The ligand and its complexes exhibit concentration-dependent cytotoxic and apoptotic behavior. The ligand induces the weakest cytotoxic and apoptotic effects on both Caco-2 cancer cells and lymphocytes. The Ni (II) complex of ligand induces high cytotoxic and apoptotic effects on both Caco-2 cancer cells and lymphocytes. The Cu (II) complex of ligand has high cytotoxic and apoptotic effects on Caco-2, but weak cytotoxic and apoptotic effects on lymphocytes. The cytotoxic and apoptotic effects of the ligand and its Ni (II) and Cu (II) complexes were found to be concentration dependent, i.e. the higher the concentration is the more cytotoxic it will be. The present findings suggest that Cu (II) complex has the potential to act as a promising anti-cancer compound against Caco-2 colon cancer cells.

KEYWORDS
apoptosis, cytotoxic effect, DNA diffusion assay, metal complexes, *vic*-dioxime ligand

1 | INTRODUCTION

Cancer is a major public health problem worldwide and is the second leading cause of death around the world. The continuous decline in cancer death rates over decades resulted in an overall drop of 26%, resulting in approximately 2.4 million fewer cancer deaths during this time period.[1] Cancer occurs as a result of contributions from environmental and genetic factors. These factors lead to disturbance in the genomic composition of a cell. The change in the cellular genome distorts the normal physiology of the cells, for example, cell division, cell differentiation, angiogenesis and cell migration, to cause cancer.[2] Although a number of methodologies have been developed and are developing to combat various types of cancer, none of the routes/drugs is totally

effective and safe. Current anti-cancer drugs have a variety of side-effects. An important restrictive factor of chemotherapy is that this treatment also affects healthy cells of the organism. Furthermore, present chemotherapy drugs have undesirable side-effects.[3] Therefore, there is extensive research for new molecules that may be used as an optimal treatment for cancer.[4] Oximes and hydrazones are categories of Schiff base compounds, and are an interesting class of coordinating ligands possessing robust applications in analytical, biological and chemical fields.[5] One of the striking features of this class of ligands and its complexes is their resemblance with bio-molecules like proteins and nucleic acids. Hydrazones have a wide variety of applications, such as hole transporting agents[5] in organic photoconductor layers, in the pharmaceutical industry as drugs for the treatment of cancer, schizophrenia, leprosy, etc.[6,7] They also have various biological activities, such as anti-microbial, analgesic, anti-inflammatory, anti-convulsant, anti-urease and anti-tumor activities.[8–10]

Hydrazone and oxime ligands include a number of probable bonding sites, such as carbonyl oxygen, imine and azomethine nitrogen atoms. Therefore, these ligands and their coordination compounds have been extensively studied.[11–14] Especially, hydrazones possessing an azomethine proton (-NHN=CH-) constitute an important class of compounds that are extensively used and studied in the area of novel drug development. It is shown that hydrazone derivatives and its complexes inhibit DNA synthesis and cell growth.[15,16]

Dioxime ligands are known to coordinate metal ions as neutral dioximes and also as monoanionic dioximates via dissociation of one oxime proton; they are also known to act as bridging ligands via coordination through the oxygens.[17,18] The chemistry of the bis-dioximate complexes of transition metal ions has attracted much attention because of their importance as reference models for vitamin B12,[19,20] dioxygen carriers,[21] catalysis in chemical transformations,[22] intramolecular hydrogen bonding and metal–metal interaction.[23,24] The exceptional stability and unique electronic properties of the complexes can be attributed to their planar structure, which is stabilized by hydrogen bonding. The high stability of complexes prepared from *vic*-dioxime ligands has been used extensively for various purposes.[25] It is well known that *vic*-dioximes and their hydrazone derivatives are capable of forming complexes with almost all the metal ions because of the convenient synthetic procedures and easy work-up. The preparation and characterization of complexes of different metal ions with *vic*-dioximes have already been reported.[26–28] Interestingly, 1:2 (metal–ligand ratio) complexes of the oxime and hydrazone derivatives of oxime are also known.[29] Previous studies reported that different derivatives of dioximes and their metal complexes possess anti-tumor activities against various cancer cell lines.[30,31] So, in continuation of our work on hydrazones, we investigated the cytotoxic and apoptotic effects of *vic*-dioxime ligand, (1E,2E)-2-(hydroxyimino)-*N*′-[(1E)-2-oxo-2phenylethylidene] ethanehydroximohydrazide (LH_2), and its Cu (II) and Ni (II) complexes on Caco-2 heterogeneous human epithelial colorectal adenocarcinoma cells, and compared them with those of anti-cancer drug Campto. Because the current drugs used for cancer treatment also affect healthy cells of the organism, human peripheral lymphocytes were used as control (healthy) cells in the present study.

2 | EXPERIMENTAL

All of the starting materials and reagents were of standard analytical grade obtained from Merck, Fluka and Aldrich, and used without further purification. The chemicals dimethylsulfoxide (DMSO), Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), RPMI 1640 medium, Histopaque-1077, phosphate-buffered saline (PBS), EDTA, SLS, Tris–HCl and NaOH were purchased from Sigma Aldrich (St Louis, MO, USA). YOYO-1 iodide was purchased from Thermo Fisher Scientific (Massachusetts, USA). High-resolution agarose and SFR agarose were purchased from Thomas Scientific (New Jersey, USA). Caco-2 cells were purchased from American Type Cell Culture Collection (ATCC HTB-37).

The melting points of the starting materials were determined on a Buchi SPM-20 apparatus in a sealed capillary and are uncorrected. Infrared (IR) spectra were obtained on a Varian 900 Fourier transform-infrared (FT-IR) spectrometer using KBr pellets. ^{1}H-NMR and ^{13}C-NMR spectra were recorded at room temperature on a Bruker 400 MHz spectrometer in DMSO. The absorption spectra were taken on a Shimadzu UV-1601 spectrophotometer. Elemental analyses were obtained using a Leco CHNS-932 analyzer. An Orion Expandable Ion Analyzer EA 940 was used for the pH measurements. The formula weights, colors, yields, melting point and molar conductance magnetic susceptibilities were determined, and elemental analyses UV–Vis, FT-IR, ^{13}C-NMR, ^{1}H-NMR of the ligand and its coordination complexes were performed.

2.1 | Synthesis of the precursor, GH_2

The starting material (1*Z*,2*E*)-2-(hydroxyimino) ethanehydroximohydrazide (GH_2)[32] was synthesized by reacting the (1*Z*,2*E*)-*N*-hydroxy-2-(hydroxyimino)

ethanimidoyl chloride[33] and hydrazinium hydroxide. Because the precursor, GH_2, was not stable at room temperature, it was used as obtained without further purification.

2.2 | Synthesis of the ligand (LH_2)

The ligand LH_2, (1E,2E)-2-(hydroxyimino)-*N*′-[(1E)-2-xo-2-phenylethylidene] ethanehydroximohydrazide was prepared by reacting (1*Z*,2*E*)-2-(hydroxyimino) ethanehydroximohydrazide (GH_2) and phenylglyoxal monohydrate in 1:1 molar ratio (Scheme 1). Figure 1 shows the tautomeric forms of the ligand, LH_2. The ligand is soluble in common solvents such as CH_2Cl_2, $CHCl_2$, DMF, EtOH and DMSO.

The metal complexes of the ligand were prepared under similar conditions in the presence of a strong base. In general, the reaction of the LH_2 with metal salts was quick and high yielding, generating mononuclear complexes corresponding to the general formula ML_2. Figure 2 represents the proposed square-planar structure for Ni (II) and Cu (II) complexes.[32]

2.3 | Cell culture and cytotoxicity assay

Human colon cancer cells (Caco-2; ATCC HTB-37) and human peripheral lymphocytes were used in the cytotoxicity assay. Caco-2 cells were cultured in DMEM, supplemented with 20% FBS and penicillin–streptomycin under humidified atmosphere of 5% CO_2 at 37°C until confluent. The cells were trypsinized, and cytotoxicity assays were carried out in 24-well plates. Caco-2 cells were plated as 5×10^4 cells per plate in 24-well plates and incubated for 24 hr, during which a partial monolayer formed. Cells were then treated with 10, 20 and 40 μM ligand and its Cu (II) and Ni (II) complexes. Cells were also treated with the *vic*-dioxime ligand and its Cu (II) and Ni (II) complexes in combination with Campto (40 μg mL^{-1}) for 24 hr.

Human peripheral lymphocytes, used as the control (healthy) cells in the experiments, were isolated using Histopaque-1077 and cultured according to Boyum, 1968.[34] Blood was diluted 1:1 with PBS and layered onto the Histopaque in the ratio of 4:3 (blood + PBS: Histopaque). The blood was centrifuged at 400 ***g*** for 30 min at room temperature. The lymphocyte layer was removed and washed twice in PBS at 250 ***g*** for 10 min. Then, the cells were washed with RPMI-1640 media. Each culture contained 1×10^3 cells mL^{-1}. Isolated lymphocytes were also treated with 10, 20 and 40 μM ligand and its Cu (II) and Ni (II) complexes. Control cells received only maintenance medium. Furthermore, the anti-cancer agent Campto (40 μg mL^{-1}) was used as the positive control, and 0.1% DMSO was used as the solvent control. The plates were incubated at 37°C in a humidified incubator with 5% CO_2 for 24 hr. At the end of the treatment time, cell viability was determined by the MTT assay.[35] The MTT assay is a colorimetric assay for assessing cell metabolic activity. NAD(P)H-dependent cellular oxidoreductase enzymes may, under defined conditions, reflect the number of viable cells present. These enzymes are capable of reducing the tetrazolium dye MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] to its insoluble formazan, which has a purple color.[35]

Briefly, the cell culture was washed with 1 × PBS and then 10 μL MTT solution was added to the culture. The cultures were incubated at 37°C for 3 hr. The whole MTT was removed with 1 × PBS, and 100 μL MTT solubilization solution was added to each culture and incubated at room temperature for 30 min until the cells were lysed and color was obtained. The absorbance was read at 570 nm by using a microplate reader (Thermo Labsystems Multiscan Spectrum).

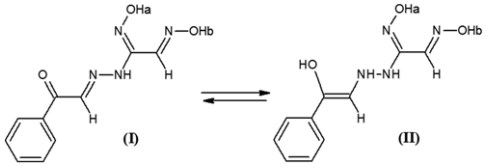
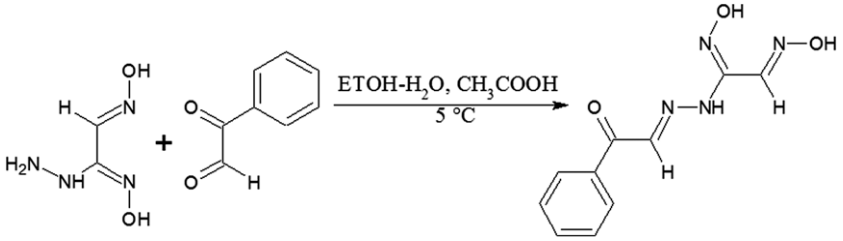


FIGURE 1 Tautomeric forms of the ligand (LH_2)

2.4 | DNA diffusion assay for apoptotic and necrotic cell death evaluation

For the evaluation of the type of cell death after treatments of *vic*-dioxime ligand and its metal complexes,



SCHEME 1 (1*E*,2*E*)-2-(hydroxyimino)-*N*′-[(1*E*)-2-oxo-2-phenylethylidene] ethanehydroximohydrazide (LH_2)

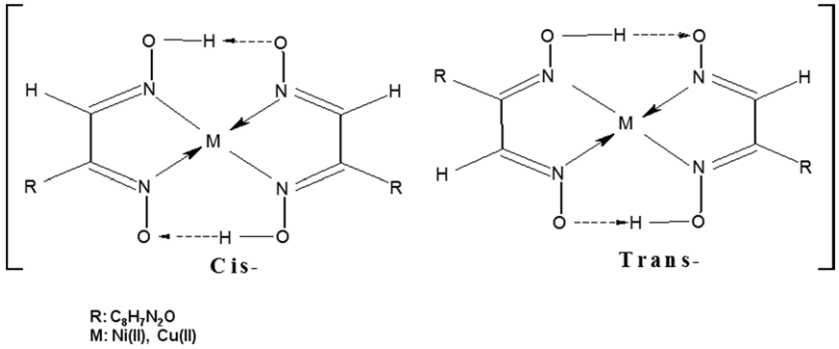


FIGURE 2 A suggested structure formulae of the square-planar complex of the ligand

DNA diffusion assay was performed following the protocol described by Singh (2005).[36] Agarose pre-coated slides were made by spreading 50 μL of 1% 3:1 high-resolution agarose on each slide, and drying them at room temperature. Microgels were made on agarose pre-coated slides by mixing extract-treated cells with 50 μL 0.6% high-resolution agarose, and pipetting this mixture onto the slide. The gel was immediately covered with 24 × 50 mm cover glasses. The slides were coded and allowed to solidify for 5 min at room temperature. The cover glasses were removed from the second layer of microgel, and 200 μL of 2% SFR agarose was layered to make a third layer, and immediately covered with 24 × 50 mm cover glasses. After 2 min, cover glasses were removed, and slides were immersed in a freshly made lysing solution (1.24 M NaCl, 1 mM tetra sodium EDTA, 5 mM Tris–HCl, 0.01 sodium lauryl sarcosine, 0.2% DMSO freshly added, 0.3 N NaOH freshly added) for 10 min at room temperature. After lysis, the slides were twice immersed in a neutralizing solution (50% ethanol, 1 mg mL^{-1} spermine, 20 mM Tris–HCl, pH 7.4) for 30 min at room temperature. The slides were air-dried and stored at room temperature. The slides were stained with 50 μL of 1 μM YOYO-1 and covered with a cover slip. One-thousand cells per slide were analysed. The cells that were undergoing apoptosis or necrosis were distinguished from normal cells. Apoptotic cell nuclei have a hazy or undefined outline without any clear boundaries due to nucleosomal-sized DNA diffusing into the agarose. Necrotic cell nuclei are bigger and poorly defined. They have a clear, defined outer boundary of the DNA halo and a relatively homogeneous halo appearance.[36]

2.5 | Statistical analysis

Each experiment was carried out triplicate. Values are expressed as means ± SD, and analyzed by one-way ANOVA (SPSS 13.0 software package program). Statistically significant difference was considered at the level of $P < 0.05$. The normality of variables was evaluated using the Kolmogorov–Smirnov *Z*-test. The statistical differences between the control and treatment groups were carried out using the non-parametric Mann–Whitney test (for independent samples). The correlations between different variables were determined using the Spearman Rank Correlation Test.

3 | RESULTS AND DISCUSSION

The starting material (1*Z*,2*E*)-2-(hydroxyimino) ethanehydroximohydrazide (GH_2)[32] for this study was prepared by reacting the (1*Z*,2*E*)-*N*-hydroxy-2-(hydroxyimino) ethanimidoyl chloride and hydrazinium hydroxide.[33] The compound (GH_2) was not stable at room temperature. Therefore, the compound (GH_2) was used as obtained without further purification. This *vic*-dioxime ligand, LH_2, containing the hydrazone group was obtained by the reaction of (1*Z*,2*E*)-2-(hydroxyimino) ethanehydroximohydrazide (GH_2)[32] and phenylglyoxal monohydrate in 1:1 molar ratio. Figure 1 shows the tautomeric forms of the ligand, LH_2. The metal complexes are intensely colored and stable in air. Figure 2 represents the proposed square-planar structure for Ni (II) and Cu (II) complexes. The metal complexes are intensely colored and stable in air. The results of the compositional and spectroscopic analyses are shown in Tables 1–4.

3.1 | NMR spectra

In the ^{1}H-NMR spectra, the OH protons of the oxime groups are not equivalent, hence two peaks for these protons are observed.[37,38] The characteristic oxime OH proton is observed in between δ 14.11 and 11.82 ppm. These chemical shifts are characteristic for hydrazones and oximes.[39,40] On the basis of ^{1}H-NMR spectra, it can be suggested that LH_2 has the (E,E)-structure. The deuterium-exchangeable protons of =N-OH groups show chemical shifts at δ 8.54 and 9.32 ppm. These singlets also indicate the (E,E)-structure of *vic*-dioximes.[41,42] The other singlet observed at δ 11.61 ppm can be assigned to

TABLE 1 Analytical and physical results for the newly synthesized compounds

Compound	Color	μ_{eff} (BM)[a]	M.p. (d)[b] (°C)	Λ^{c}_{M}	Yield (%)	Elemental analyses (%) (calcd/found) C	H	N	M
$C_{10}H_{10}N_4O_3$ (LH_2)	Yellow	–	127	1.3	77	51.28 (51.52)	4.30 (4.20)	23.92 (23.28)	–
$C_{20}H_{18}N_8O_6Ni$	Dark-brown	Dia.	210	7.4	52	45.67 (43.57)	3.43 (3.47)	21.32 (21.28)	11.23 (11.51)
$C_{20}H_{18}N_8O_6Cu$	Dark- green	1.63	208	6.3	73	45.29 (44.94)	3.40 (3.74)	21.14 (20.79)	11.98 (12.33)

[a]μ_{eff}: magnetic moment; Dia.: diamagnetic.

[b]d: decomposition.

[c]Molar conductivity (Ω^{-1} cm^2 mol^{-1}).

TABLE 2 ^{1}H-NMR spectra of the newly synthesized ligand and Ni (II) complex in DMSO-d^6 (δ, ppm)

Compounds	O-H .... O[a]	O-H[a]	N-H[a]	CH=NOH	$CH_{Arom.}$
LH_2	–	14.11–11.82 (1H each, 2s)	11.61 (1H, s)	8.54–9.32 (1H each, 2s)	7.48 2H, d, J = 8.65 8.22 2H, d, J = 8.65
$(L^1H)_2Ni$	15.32 (2H, s)	–	–	–	–

the -N-H signal, which can also be easily identified by deuterium-exchange (Table 2). The aromatic protons would resonate as multiples between δ 7.48 and 8.22 ppm. In the ^{1}H-NMR spectra of LH_2, two absorption bands, which are assigned to the oxime -O-H protons, appear at a lower field, at δ 14.11–11.82. The chemical shifts belonging to the -O-H protons in *vic*-dioxime disappeared in the ^{1}H-NMR spectrum of the Ni (II) complex, and the presence of a new resonance at a lower field at δ 15.32 ppm was assigned by formation of the hydrogen bridge, which could easily be identified by deuterium-exchange.[43] The geometric isomers of Ni (II) complex can be inferred from the ^{1}H-NMR spectra, as the alternative chemical environment will show two O···H–O bridge protons in the *cis*-form, but also one ^{1}H-NMR resonance in the *trans*- structure. The observed spectrum has only one ^{1}H-NMR resonance, suggesting the *trans*- of the complexes.[43]

A ^{13}C-NMR spectrum is one of the most convenient ways to prove the true structure of *vic*-dioxime. In the ^{13}C-NMR spectrum of LH_2, the carbon resonances of the oxime groups are observed at 146.57 and 145.79 ppm. Observation of *vic*-dioxime and -O-H protons in the ^{1}H-NMR, and of dioxime carbons in the ^{13}C-NMR spectra at two different frequencies in each case, indicates that the *vic*-dioxime has an *anti*-structure.[44] The equivalent carbon atoms, especially the adjacent hydroxyimino groups, also confirm the (E,E)-structure of *vic*-dioximes.[45]

3.2 | Fourier transform-infrared spectra

The tentative assignments of the most characteristic IR bands were observed and are given in Table 3. The FT-IR spectrum of the -O-H group of oxime shows stretching vibrations at 3450 and 2600 cm^{-1} instead of a band at about 3230 cm^{-1} because of an intermolecular hydrogen bonding N···H–O.[46]

Also, the NH stretching band of LH_2 was not observed in the FT-IR spectra probably due to overlapping with the intermolecular hydrogen-bonded -O-H stretching frequency. The stretching band of the imine group -C=N of these compounds was observed at 1611 cm^{-1}. The stretching belonging to the -NH and -N-O groups occur at 3420 and 1005 cm^{-1}, respectively. These values are in agreement with the previously reported hydrazone and oxime derivatives.[39,40] The FT-IR spectra of Ni (II) and Cu (II) have been compared with those for similar compounds in order to establish the coordination modes of the oxime ligands in these complexes.[32,43] The more relevant -O-H, -C=N and -N-O absorption bands are given in Table 3. The Ni (II) and Cu (II) complexes have FT-IR spectra very similar to those of the free ligand, except for the absence of the -O-H stretching vibrations. A weak and low-intensity band at 2340–2170 cm^{-1} indicates the formation of the hydrogen bridge during complexation of the ligand, as the hydrogen bridge reduces the strength of the -O-H bond. The occurrence of hydrogen bridges by the loss of one oxime proton per oxime molecule during the complexation of this ligand accounts for the two non-identical =N-C- linkages, -C=N-O-H in the complexes.[47] The stretching vibrations of the azomethine groups appearing at 1610 cm^{-1} in the free ligand are shifted to 1545–1540 cm^{-1} in the mononuclear Ni (II) and Cu (II) complexes. At the same time, in the complexes the bands observed near 1005 cm^{-1} in the free ligand, which are assigned to the ν(N-O), are shifted to lower frequencies (990–980 cm^{-1}) after complexation.

TABLE 3 Characteristic FT-IR bands (cm^{-1}) of the newly synthesized compounds as KBr pellets

Comp.	ν (NH)	ν (OH)	ν ($CH_{ar.}$)	ν (OH..O)	ν($C=N_{oxime}$)	ν($C=N_{hyd.}$)	ν (NO)	ν(C=O)
LH_2	3420 (b)	3230 (b)	3055 (w)	–	1610 (s)	1540 (m)	1005 (s)	1670 (m)
$(L^1H)_2Ni$	3245 (b)	–	3055 (w)	2170 (w)	1545 (s)	1590 (m)	980 (s)	–
$(L^1H)_2Cu$	3440 (b)	3340 (b)	3065 (w)	2340 (w)	1540 (s)	1595 (m)	990 (s)	–

b = broad, s = strong, m = medium, w = weak.

TABLE 4 Characteristic UV–Vis bands of the newly synthesized compounds

Compounds	Solvent	Wavelength, λ_{max} (nm)
LH_2	ETOH	345, 295
$(L^1H)_2Ni$	DMSO	678, 546, 488, 350
$(L^1H)_2Cu$	DMSO	637, 450, 325, 280

3.3 | Electronic spectra

The electronic spectra of the ligand and its complexes were recorded in ethanol and DMSO (Table 4). In the electronic spectra of the ligand and its metal complexes, the 280–350 nm band seems to be due to both the $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions of -C=N and charge-transfer transition arising from p electron interactions between the metal and ligand, which involve either a metal-to-ligand or ligand-to-metal electron transfer.[48] The electronic spectra of the Ni (II) and Cu (II) complexes show absorption bands at 678 and 637 nm attributed to the $^1A_{1g} \rightarrow {}^1B_{1g}$ for Ni (II) complex and $^2T_{2g} \rightarrow {}^2E_g$ for Cu (II) complex transitions, which are compatible with the complexes having a square-planar structure.[49]

3.4 | Magnetic susceptibility

The structures of the mononuclear complexes are supported by magnetic moment data. The Ni (II) is diamagnetic as expected for d^8 electronic configuration.[30] The Cu (II) complex is paramagnetic and their magnetic moment is 1.63 BM. The alternative chemical environments will give two (O···H–O) bridge protons in the *cis*-form, but only one in the *trans*-form. Observation of the (O···H–O) in the IR spectrum and ^{1}H-NMR spectra, at one frequency in each case, indicate that the Ni (II) complex is in the *anti*-form media. According to the above results, square-planar geometry for the Ni (II) and Cu (II) complexes is proposed.[32,43,44]

3.5 | Molar conductance

As seen from Table 1, the molar conductance measured in DMSO solution (about 1×10^{-3} M) for the ligand and its mononuclear complexes is in the range of 1.3, 7.4–6.3 $\Omega^{-1}\,cm^{-2}\,mol^{-1}$. This result shows that the complexes are non-electrolytes.[43]

3.6 | Cytotoxic effect of ligand and its Cu (II) and Ni (II) complexes

The cytotoxic effects of the *vic*-dioxime ligand and their Ni (II), Cu (II) complexes were analyzed by culturing the cells of human epithelial colorectal adenocarcinoma (Caco-2) and human peripheral lymphocytes *in vitro*. The Caco-2 cell line is homogeneous cells originally derived from a colon carcinoma. The results of the cytotoxicity test (MTT assay) are presented in Table 5. The *vic*-dioxime ligand induced a degree of cytotoxic effect on the Caco-2 cells and peripheral lymphocytes, but this effect is not statistically significant. The ligand and Campto combination induced higher cytotoxic effects on Caco-2 cells and lymphocytes when compared with both the control and solvent control.

The Cu (II) complex of the ligand was significantly increased with the cytotoxic effect dependent on increased concentration in Caco-2 cells. The Cu (II) complex of the ligand did not cause a cytotoxic effect on peripheral lymphocytes. The Cu (II) complex + Campto combination induced a very high cytotoxic effect on Caco-2 cells when compared with control and solvent control. The cytotoxic effect on lymphocytes was found to be statistically significantly increased compared with control and solvent control. Generally, Cu (II) of ligand complex + Campto inhibited cell proliferation more than positive control (Campto), and showed a higher cytotoxic effect than Campto at both treatment periods. Increasing concentrations caused a significant increase of cytotoxic effects on peripheral lymphocytes. Also, the positive control Campto induced cytotoxic effects on peripheral lymphocytes (Table 5).

The Ni (II) complex of ligand induced significant cytotoxic effects on Caco-2 cells at 40 μM concentration (61.90%), and a low cytotoxic effect at 10 μM concentration (12.50%). The cytotoxic effect on lymphocytes was found to be higher when compared with ligand. The combination of Ni (II) complex + Campto induced a very

TABLE 5 The cytotoxic effect of *vic*-dioxime ligand on human colon cancer cells (Caco-2) and peripheral blood lymphocytes

	% Cytotoxicity (± SD)	
Groups	**Caco-2 cells**	**Lymphocytes**
Control	0.00 ± 0.00	0.00 ± 0.00
Solvent control (DMSO) (% 0.1)	2.13 ± 0.179	2.21 ± 0.024
Campto (40 μg mL^{-1})	55.36 ± 0.026*	23.52 ± 0.015*
LH_2–1	8.18 ± 0.090	2.00 ± 0.047
LH_2–2	16.67 ± 0.020*	3.76 ± 0.031
LH_2–3	24.39 ± 0.073*	5.82 ± 0.019
LH_2–1 + Campto	56.21 ± 0.144*	51.17 ± 0.007*
LH_2–2 + Campto	60.61 ± 0.044*	52.58 ± 0.033*
LH_2–3 + Campto	65.76 ± 0.031*	53.52 ± 0.021*
LH_2-Cu1	66.61 ± 0.007*	3.12 ± 0.039
LH_2-Cu2	70.18 ± 0.008*	5.16 ± 0.085
LH_2-Cu3	76.13 ± 0.002*	24.41 ± 0.031*
LH_2-Cu1 + Campto	86.42 ± 0.001*	49.77 ± 0.001*
LH_2-Cu2 + Campto	90.32 ± 0.006*	51.64 ± 0.007*
LH_2-Cu3 + Campto	92.54 ± 0.041*	52.58 ± 0.041*
LH_2-Ni1	12.50 ± 0.022	8.92 ± 0.046
LH_2-Ni2	26.49 ± 0.049*	13.15 ± 0.036
LH_2-Ni3	61.90 ± 0.012*	36.15 ± 0.030*
LH_2-Ni1 + Campto	56.25 ± 0.013*	23.00 ± 0.029*
LH_2-Ni2 + Campto	77.08 ± 0.005*	38.03 ± 0.010*
LH_2-Ni3 + Campto	82.74 ± 0.017*	46.48 ± 0.002*

*$P < 0.05$ [**LH_2–1**: 10 μM ligand; **LH_2–2**: 20 μM ligand; **LH_2–3**: 40 μM ligand; **LH_2-Cu1**: 10 μM Cu (II) complex; **LH_2-Cu2**: 20 μM Cu (II) complex; **LH_2-Cu3**: 40 μM Cu (II) complex; **LH_2-Ni1**: 10 μM Ni (II) complex; **LH_2-Ni2**: 20 μM Ni (II) complex; **LH_2-Ni3**: 40 μM Ni (II) complex].DMSO, dimethylsulfoxide.

high cytotoxic effect on Caco-2 cells when compared with control, solvent control and Campto. Also, the cytotoxic effect on lymphocytes was found to be statistically significantly increased compared with control, solvent control and Campto. These results are also statistically significant (Table 5). The present results reveal that Cu (II) complex + Campto and the Ni (II) complex + Campto showed cytotoxic effects on Caco-2 cells and lymphocytes, but the Ni (II) complex + Campto has a less cytotoxic effect compared with the Cu (II) complex + Campto against lymphocytes.

Different biological activities like DNA binding and cleavage studies of copper and nickel complexes containing ferrocenyl hydrazone ligands were performed by Krishnamoorthy *et al.* (2011).[50] They evaluated the DNA binding, DNA cleavage, protein binding and *in vitro* cytotoxic activities of bivalent transition metal hydrazone complexes of cobalt, nickel and copper. Complexes interacted with DNA, and intercalative mode of binding was revealed, whereas metal complexes resulted in conformational change when interacted with bovine serum albumin. The anti-cancer activity of complexes was tested against HeLa tumor cells and NIH 3 T3 normal cells. The results of the study showed that the complexes are toxic only against tumor cells but not to normal cells. Furthermore, they found that the complexes with copper ion as a metal center were more potent as compared with nickel and cobalt.[50] Li *et al.* (2011a)[51] examined the cytotoxic behavior of Mn (II) and Ni (II) complexes of thiosemicarbazones against K562 leukemia cell line, and reported that Ni complexes were more active than that of Mn. Sirajuddin *et al.* (2015)[52] observed the *in vitro* cytotoxic potential of carboxylic acid derivatives against H-157, BHK-21 and HCEC cell lines. The derivatives were non-toxic towards normal cell lines, whereas significant inhibition was found against cancer cell lines. The present results reveal that cobalt complexes are more active than Ni complexes against cancer cell lines. These complexes are more potent inhibitors than ligand against H157 cancer cells, whereas the complexes as well as ligands are non-toxic to normal cell lines. The non-toxic nature of compounds towards normal cells suggests their safe and significant selectivity index.

3.7 | Apoptotic and necrotic cell death

Chemical-induced cell apoptotic and necrotic cell death were determined usingDNA diffusion assay in this study, and the results are presented in Table 6. The assay is a simple, sensitive and reliable technique that allows the assessment of programmed cell death or necrosis events based on nuclear morphology.[53]

The *vic*-dioxime ligand did not cause an apoptotic effect on the Caco-2 cells and peripheral lymphocytes. When cells were treated with ligand and Campto together, Caco-2 cells and lymphocytes had higher apoptotic effects than control and solvent control groups. Despite their apoptotic activity on Caco-2 cells, the rate of necrotic cell death caused by ligand in both Caco-2 cells and lymphocytes was found to be low.

The Cu (II) complex of the ligand has been found to have an increased apoptotic effect depending on increased concentration of Caco-2 cells. The Cu (II) complex of the ligand did not cause a significant apoptotic effect in peripheral lymphocytes (Table 6). When Cu (II) complex was used in combination with Campto, the apoptotic effect on Caco-2 cells increased and reached

TABLE 6 The apoptotic effect of *vic*-dioxime ligand on human colon cancer cells (Caco-2) and peripheral blood lymphocytes

	Caco-2			Lymphocytes		
Groups	**Apoptotic cells (% ± SD)**	**Necrotic cells (% ± SD)**	**Total cells**	**Apoptotic cells (% ± SD)**	**Necrotic cells (% ± SD)**	**Total cells**
Control	0.00 ± 0.00	0.00 ± 0.00	3000	0.00 ± 0.00	0.00 ± 0.00	3000
Solvent control (DMSO) (% 0.1)	2.00 ± 0.10	0.10 ± 0.01	3000	2.00 ± 1.00	0.10 ± 0.10	3000
Campto (40 μg mL^{-1})	52.33 ± 0.57*	1.67 ± 0.56	3000	21.67 ± 1.54*	0.3 ± 0.06	3000
LH_2–1	6.33 ± 0.57	2.67 ± 0.56	3000	2.33 ± 0.56	0.2 ± 0.10	3000
LH_2–2	15.67 ± 0.56*	1.33 ± 0.57	3000	3.67 ± 0.57	0.5 ± 0.30	3000
LH_2–3	21.33 ± 0.57*	2.67 ± 1.54	3000	4.33 ± 0.56	0.8 ± 0.00	3000
LH_2–1 + Campto	49.67 ± 0.56*	3.33 ± 0.57	3000	41.33 ± 1.54*	4.67 ± 0.57	3000
LH_2–2 + Campto	53.33 ± 1.54*	5.67 ± 0.56	3000	41.67 ± 0.56*	5.33 ± 0.56	3000
LH_2–3 + Campto	57.67 ± 0.56*	5.00 ± 0.00	3000	42.00 ± 1.00*	6.67 ± 0.57	3000
LH_2-Cu1	61.67 ± 0.57*	2.33 ± 1.54	3000	3.00 ± 0.00	0.27 ± 0.06	3000
LH_2-Cu2	64.33 ± 1.54*	3.00 ± 1.00	3000	4.67 ± 0.56	0.33 ± 0.06	3000
LH_2-Cu3	70.00 ± 0.00*	2.67 ± 0.56	3000	17.33 ± 0.57*	0.67 ± 0.57	3000
LH_2-Cu1 + Campto	77.33 ± 2.08*	5.00 ± 1.00	3000	41.00 ± 1.00*	2.00 ± 0.00	3000
LH_2-Cu2 + Campto	81.00 ± 0.56*	3.33 ± 0.57	3000	44.67 ± 0.57*	1.33 ± 0.56	3000
LH_2-Cu3 + Campto	86.67 ± 0.57*	4.67 ± 1.54	3000	46.33 ± 0.56*	2.67 ± 0.57	3000
LH_2-Ni1	9.00 ± 1.00	3.33 ± 0.56	3000	7.00 ± 0.00	1.33 ± 1.73	3000
LH_2-Ni2	17.67 ± 0.57*	5.00 ± 0.00	3000	9.33 ± 0.56	1.67 ± 1.57	3000
LH_2-Ni3	48.33 ± 0.56*	7.67 ± 0.57	3000	31.00 ± 1.00*	2.00 ± 0.00	3000
LH_2-Ni1 + Campto	49.67 ± 1.54*	4.33 ± 1.54	3000	20.00 ± 1.00*	1.00 ± 1.00	3000
LH_2-Ni2 + Campto	65.00 ± 0.00*	6.00 ± 0.00	3000	27.67 ± 0.57*	1.00 ± 0.00	3000
LH_2-Ni3 + Campto	72.33 ± 0.56*	7.67 ± 0.56	3000	37.33 ± 0.57*	1.67 ± 1.57	3000

*$P < 0.05$ [**LH_2–1**: 10 μM ligand; **LH_2–2**: 20 μM ligand; **LH_2–3**: 40 μM ligand; **LH_2-Cu1**: 10 μM copper (II) complex; **LH_2-Cu2**: 20 μM copper (II) complex; **LH_2-Cu3**: 40 μM copper (II) complex; **LH_2-Ni1**: 10 μM nickel (II) complex; **LH_2-Ni2**: 20 μM nickel (II) complex; **LH_2-Ni3**: 40 μM nickel (II) complex].DMSO, dimethylsulfoxide.

86.67% in the 40 μM Cu (II) complex and Campto mixture group. When the Cu (II) complex of the ligand was used with Campto, the apoptotic effect on lymphocytes was found to be statistically significantly higher than control and solvent control. The rate of necrotic cell death in both Caco-2 cells and lymphocytes was found to be very low.

The Ni (II) complex of ligand has not been found to have an increasing apoptotic effect depending on concentration of Caco-2 cells and lymphocytes, but the apoptotic effect on Caco-2 cells and lymphocytes was found to be significantly high in comparison with control and solvent control. When the Ni (II) complex was used in combination with Campto, the apoptotic effect on Caco-2 cells increased and reached 72.33 ± 0.56% in the 40 μM Ni (II) complex and Campto mixture group. When the Ni (II) complex of the ligand was used with Campto, the apoptotic effect on lymphocytes was found to be statistically significantly higher than control, solvent control and Campto. The Ni (II) complex of ligand caused a high apoptotic effect both on Caco-2 cells and in lymphocytes, while necrotic cell death is observed with low levels of Ni (II) complex for Caco-2 cell line on lymphocytes.

Apoptosis and necrosis are two major mechanisms of cell death.[54] Apoptosis, in fact, is a programmed mechanism for cell death, characterized by morphological and biochemical changes. This evolutionarily conserved cell death process is important for normal development and tissue homeostasis.[55,56] It also occurs during pathological conditions, as well as in response to many cancer chemotherapeutic agents. Apoptosis is induced by a variety of stimuli, such as cytotoxic compounds.[57] Cells that are damaged by external injury undergo necrosis,

while cells that are induced to commit programmed suicide because of internal or external stimuli undergo apoptosis. An increasing number of chemo-preventive agents have been shown to stimulate apoptosis in pre-malignant and malignant cells *in vitro* or *in vivo*.[58]

The cytotoxicity of Cu (II) complexes may be caused by their ability to bind and cleave DNA, which leads to the arrest of the cell cycle and apoptosis, or the generation of reactive oxygen species, followed by cell death.[59,60] In light of the fact that copper is a bio-essential element responsible for numerous bioactivities in living organisms, (CuL2) should be an excellent anti-tumor agent for applications.[61]

vic-Dioximes are important ligands because of their considerable attention in biology and chemistry.[62] Some *vic*-dioxime ligands show anti-microbial properties.[62] Previous studies suggested that hydrazone and oxime derivatives and their metal complexes possess anti-proliferative effects against tumor cells, such as human breast cancer cells, ovarian cancer cells, renal cancer cells, hematological tumor cells, prostate cancer cells, hepatocellular carcinoma cells, lung carcinoma cells and HL-60 human promyelocytic leukemia cells.[63–67] They exhibit their anti-cancer potential as anti-proliferative, cytotoxic, cell cycle arrest at G2/M, inducing apoptosis, caspase activation, and by inhibiting tubulin polymerization.[63–67]

One of the salient characteristics of carcinogenesis is that the dividing tumor cells fail to initiate apoptosis following DNA damage.[68] Development of approaches that reinstall the apoptotic machinery selectively within tumor cells could be an effective measure of cancer control.[69] The primary mode of action of most anti-cancer drugs entails the induction of apoptosis in neoplastic cells.

Currently, cancer is the second cause of death around the world. The incidence of cancer is increasing gradually because of high population growth, and is expected to reach 75% by 2030.[70] There are numerous medications present for treatment of various cancer types, but there is not any drug that is completely effective and safe. Furthermore, most of the anti-cancer drugs affect healthy cells of patients, and produce other side-effects.[3] Therefore, researches are under way to develop new anti-cancer drugs to reduce cancer mortality rates and the costs of cancer treatment, find the best cancer treatments, and improve the lives of cancer patients. The results of the present study suggest that the Cu (II) complex of *vic*-dioxime ligand represents a potential anti-cancer agent because of its high cytotoxic and apoptotic effect against colon cancer cells, and its low effects on human peripheral blood lymphocytes. However, in order to elucidate the specific action mechanism of this complex, more detailed studies have to be carried out using different test systems.

4 | CONCLUSION

In this study, two new *vic*-dioxime derivatives containing hydrozone side groups and their metal complexes with Cu (II) and Ni (II) were synthesized and evaluated, and their cytotoxic, apoptotic and necrotic effects using MTT assay and DNA diffusion assay on Caco-2 heterogeneous human epithelial colorectal adenocarcinoma cell line and human peripheral lymphocytes were determined. As a result of this study, among the test compounds attempted Cu (II) complex of ligand showed higher cytotoxic and apoptotic activity against tested cancer cells than Ni (II) complex.

The results of the present work suggest that these compounds are effective inhibitors, and may be promising agents for anti-tumor testing with *in vivo* studies on animals under ethical regulations. However, the exact mechanism of apoptosis of the compounds under consideration in cancer cells vs. normal cells still has to be determined. These complexes may be suitable drugs, and therefore beneficial and potential alternatives, and hence should be investigated further in pharmacological studies on how this ligand and its Cu (II) complex can be used for developing new drugs for cancer treatment.

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REFERENCES

[1] R. L. Siegel, K. D. Miller, A. Jemal, *CA Cancer J. Clin.* **2018**, *68*, 7.

[2] I. Buhari, A. H. Roslida, M. T. Hidayat, A. M. Mat Jais, *J. Cancer Sci. Ther.* **2015**, *7*, 186.

[3] O. Prakash, A. Kumar, P. A. Kumar, *Am. J. Pharmacol. Sci.* **2013**, *1*(6), 104.

[4] F. E. Koehn, G. T. Carter, *Discov. Med.* **2005**, *5*(26), 159.

[5] A. Zulfikaroglu, C. Yuksektepe, H. Bati, N. Caliskan, O. Buyukgungor, *J. Struct. Chem.* **2009**, *50*, 1166. https://doi.org/10.1007/s10947-009-0171-3

[6] E. Massarani, D. T. Nardi, L. Degen, *J. Med. Chem.* **1971**, *14*(7), 633.

[7] D. Mishra, S. Naskar, A. J. Blake, S. K. Chattopadhyay, *Inorg. Chim. Acta* **2007**, *360*, 2291.

[8] M. D. Altintop, Z. A. Kaplancikli, G. A. Çiftçi, R. Demirel, *Eur. J. Med. Chem.* **2014**, *74*, 264.

[9] M. Ahmad, H. Pervez, S. Zaib, M. Yaqub, M. M. Naseer, S. U. Khan, J. Iqbal, *RSC Adv.* **2016**, *6*, 60 826.

[10] H. Pervez, M. Ahmad, S. Zaib, M. Yaqub, M. M. Naseer, J. Iqbal, *Med. Chem. Commun.* **2016**, *7*, 914.

[11] J. A. Bertrand, J. H. Smith, P. G. Eller, *Inorg. Chem.* **1974**, *13*, 1649.

[12] S. P. Wan, W. Mori, S. Yamada, *Synth. React. Inorg. Met. Org. Chem.* **1986**, *16*, 1273.

[13] M. Maekava, S. Kitagawa, Y. Nakao, S. Sakamoto, A. Yatani, W. Mori, S. Kashino, M. Munakata, *Inorg. Chim. Acta* **1999**, *293*, 20.

[14] W. Kaminsky, J. P. Jasinsky, R. Woudenberg, K. I. Goldberg, D. X. West, *J. Mol. Struct.* **2002**, *608*, 135.

[15] D. K. Johnson, T. B. Murphy, N. J. Rose, W. H. Goodwin, L. Pickart, *Inorg. Chim. Acta* **1982**, *67*, 159.

[16] M. Alam, S. A. A. Nami, A. Husain, D. U. Lee, S. Park, *C. R. Chim.* **2013**, *16*(3), 201.

[17] D. Burdinski, F. Birkelbach, T. Weyhermuller, U. Florke, H. J. Hampt, M. Lengen, A. Trautwein, E. Bill, K. Weighardt, P. Chaudhuri, *Inorg. Chem.* **1998**, *37*, 1009.

[18] R. Ruiz, M. Julve, J. Faus, F. Lioret, M. C. Munoz, Y. Journaux, C. Bois, *Inorg. Chem.* **1997**, *36*, 343.

[19] N. B. Pahor, R. Dreos-Garlatti, S. Geremia, L. Randaccio, G. Tauzher, E. Zangrando, *Inorg. Chem.* **1990**, *29*, 3437.

[20] N. B. Pahor, M. Forcolin, L. Marzilli, G. L. Randaccio, M. F. Summers, P. J. Toscano, *Coord. Chem. Rev.* **1985**, *63*, 1.

[21] K. A. Lance, K. A. Goldsby, D. H. Busch, *Inorg. Chem.* **1990**, *29*, 4537.

[22] M. Kijma, K. Miyamori, T. Nakamura, T. Sato, *Bull. Chem. Soc. Jpn.* **1990**, *63*, 2549.

[23] A. Yari, S. Azizi, A. Kakanejadifard, *Sensor Actuators B Chem.* **2006**, *119*, 167.

[24] A. Das, S. Peng, S. Bhattacharya, *Polyhedron* **2001**, *20*, 327.

[25] M. Kurtoğlu, M. M. Dağdelen, S. Toroğlu, *Transition Met. Chem.* **2006**, *31*, 382.

[26] E. Canpolat, M. Kaya, *J. Coord. Chem.* **2002**, *55*(8), 961.

[27] M. Kurtoğlu, *Synth. React. Inorg. Met. Org. Nano Metal Chem.* **2004**, *34.5*, 967.

[28] N. Sarıkavaklı, G. İrez, *Turk. J. Chem.* **2005**, *29*, 107.

[29] S. Nigam, M. M. Patel, A. Ray, *J. Phys. Chem. Solids* **2000**, *61*(9), 1389.

[30] E. M. Jouad, M. Allain, M. A. Khan, G. M. Bouet, *Polyhedron* **2005**, *24*, 327.

[31] H. Beraldo, R. Lima, L. Teixeira, A. Moura, D. West, *J. Mol. Struct.* **2000**, *553*, 43.

[32] N. Sarıkavaklı, *J. Instr. Sci. Technol.* **2017**, *4*(161), 7.

[33] H. Britzinger, R. Titzmann, *Ther. Ber.* **1952**, *85*, 345.

[34] A. Boyum, *J. Clin. Lab. Invest. Suppl.* **1968**, *21*, 77.

[35] T. Mossman, *J. Immunol. Methods* **1983**, *65*, 55.

[36] N. P. Singh, *Methods Mol. Med.* **2005**, *111*, 55.

[37] E. Taş, A. Cukurovali, M. Kaya, *J. Coord. Chem.* **1998**, *44*(1–2), 109.

[38] H. I. Uçan, R. Mirzaoğlu, S. React, *Inorg. Met. Org. Chem.* **1990**, *20*, 437.

[39] K. H. Reddy, N. B. L. Prasad, T. S. Reddy, *Talanta* **2003**, *59*(3), 425.

[40] R. Gup, M. Serin, G. Karayel, *Chem. Pap.* **2007**, *61*(4), 1.

[41] V. Alexander, *Inorg. Chim. Acta* **1989**, *163*, 25.

[42] V. A. Alexander, *Inorg. Chim. Acta* **1993**, *204*, 109.

[43] E. Canpolat, M. Kaya, *Transition Met. Chem.* **2004**, *29*, 550.

[44] E. Taş, M. Ulusoy, M. Güler, I. Yılmaz, *Transition Met. Chem.* **2004**, *29*(2), 180.

[45] L. J. Kirschenbaum, R. K. Panda, E. T. Borish, E. Mentasti, *Inorg. Chem.* **1989**, *28*, 3623.

[46] M. A. Deveci, G. İrez, *Synth. React. Inorg. Met. Org. Chem.* **1996**, *26*(5), 871.

[47] M. S. Hussain, H. M. Al-Mohdar, A. R. Al-Arfaj, *J. Coord. Chem.* **1988**, *18*, 339.

[48] N. Sarıkavaklı, H. T. Çakıcı, *Asian J. Chem.* **2012**, *24.6*, 2643.

[49] N. V. Thakkar, R. M. Patil, *Synth. React. Inorg. Met, Org. Chem.* **2000**, *30*(6), 1159.

[50] P. Krishnamoorthy, P. Sathyadevi, A. H. Cowley, R. R. Butorac, N. Dharmaraj, *Eur. J. Med. Chem.* **2011**, *46*, 3376.

[51] M. X. Li, L. Z. Zhang, D. Zhang, B. S. Ji, J. W. Zhao, *Eur. J. Med. Chem.* **2011**, *46*, 4383.

[52] M. Sirajuddin, S. Ali, V. McKee, H. Ullah, *Spectrochim. Acta, Part A* **2015**, *138*, 569.

[53] A. Macovei, M. Donà, D. Carbonera, A. Balestrazzi, in *Methods in Molecular Biology*, (Eds: L. De Gara, V. Locato), Humana Press, New York, NY **1743** 2018, 107.

[54] A. Hussain, M. F. Alajmi, M. T. Rehman, A. A. Khan, P. A. Shaikh, R. A. Khan, *Molecules* **2018**, *23*, 1232.

[55] T. S. Zheng, T. Schlosser, R. Dao, R. Hingorani, I. N. Crispe, J. L. Boyer, R. A. Flavell, *Proc. Natl Acad. Sci. USA* **1998**, *95*, 13 618.

[56] F. Liang, C. Wu, H. Lin, T. Li, D. Gao, Z. Li, J. Wei, C. Zheng, M. Sun, *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2469.

[57] M. M. Barry, C. A. Behnde, A. Eastman, *Biochem. Pharmacol.* **1990**, *40*, 2353.

[58] S. Y. Sun, N. Hail Jr., R. Lotan, *J. Natl Cancer Inst.* **2004**, *96*, 662.

[59] E. R. Jamienson, S. J. Lippard, *Chem. Rev.* **1999**, *99*(9), 2467.

[60] S. Tardito, O. Bussolati, M. Maffini, M. Tegoni, M. Giannetto, V. Dall'asta, R. Franchi-Gazzola, M. Lanfranchi, M. A. Pellinghelli, C. Mucchino, G. Mori, L. Marchio, *J. Med. Chem.* **2007**, *50*(8), 1916.

[61] J. Xie, Z. Cheng, W. Yang, H. Liu, W. Zhou, M. Li, Y. Xu, *Appl. Organomet. Chem.* **2015**, *29*, 157.

[62] İ. Babahan, E. Poyrazoğlu Çoban, A. Özmen, H. Bıyık, B. İşman, *Afr. J. Microbiol. Res.* **2011**, *5*(3), 271.

[63] R. W. Fingberg, R. C. Moellering, F. P. Tally, *Clin. Infect. Dis.* **2004**, *39*, 1314.

[64] G. N. Ling, *Physiol Chem Phys Med NMR* **1986**, *18*, 3.

[65] S. Rollas, Ş. G. Küçükgüzel, *Molecules* **2007**, *12*, 1910.

[66] L. W. Zheng, L. L. Wu, B. X. Zhao, W. L. Dong, J. Y. Miao, *Bioorg. Med. Chem.* **2009**, *17*(5), 1957.

[67] M. Arshad, M. S. Khan, S. A. A. Nami, D. Ahmad, *Russ. J. Gen. Chem.* **2018**, *88*, 2154.

[68] C. B. Thompson, *Science* **1995**, *267*, 1456.

[69] S. R. Denmeade, J. T. Isaacs, *Cancer Control* **1996**, *3*, 303.

[70] World Health Organization. Global Health Observatory (GHO) data. NCD mortality and morbidity. **2013**. http://www.who.int/gho/ncd/mortality_morbidity

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